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DONOVANOSIS: A MORPHOLOGIC STUDY*

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ABSTRACT

Morphologic studies were made of specimens from a case of granuloma inguinale. The microorganism and the abnormalities of the microvasculature were readily identifiable by light microscopy in plastic-embedded material. The bacteria were predominantly found within vacuoles of histiocyte-like cells. Wide differences in the morphologic condition of the phagocytized organisms were evident, an observation considered to reflect degrees of degradation.

Vesicle formation was commonly seen in association with the bacterial wall. These spheres were extrabacterial and no granulations were noted within either the vesicles or the bacterial cytoplasm. The absence of "dense cored" structures offered no morphologic support for the presence of "bacteriophage-like" entities.

The microvasculature was characterized by distended organelles within the endothelial and pericytic cytoplasm.

Granuloma inguinale was first evaluated morphologically by Donovan [1] through the use of tissue smear preparations. His findings have been extended to further implicate *Calymmatobacterium granulomatis* (*Donovania granulomatis*) with the pathogenesis of the disease [2]. Standard light microscopy (i.e., H & E staining of formalin-fixed tissue) usually provides unsatisfactory tissue/organism relationships; however, Donovan bodies (the intracellular microorganisms) are evident in Wright–Giemsa-stained crushed tissue and in Warthin–Starry silver-stained sections [3]. Ultrastructural studies of this disease have been reported by Davis [3], and Davis and Collins [4] who emphasized the morphologic characteristics of the microorganism.

In this paper we will present both the relationship of the organism within the tissue as well as the other structural elements in the diseased area. Furthermore, the diagnostic value of light microscopy of the diseased tissue which has been glutaraldehyde fixed and plastic embedded will be discussed. This method gives cytologic detail not possible with formalin-fixed/wax-embedded sections and has been reported in some pathologic conditions to allow a more accurate and possibly earlier diagnosis [3, 5].

The case report on the patient will be the subject of a future presentation.

MATERIALS AND METHODS

Tissue was biopsied from the perimeter of an extensive, clinically typical, inguinal lesion, cut into small (1–2 mm^3) blocks and immersed in 3% glutaraldehyde 0.1 M phosphate fixative. After a 24-hr period at room temperature, the tissue was rinsed twice in phosphate buffer. The samples were postfixed in a 1% osmium tetroxide 0.1 M phosphate system for 4 hr. Dehydration was carried out in cold ethanol followed by embedding in Maraglas-732 epoxy media [6].

Thin sections (light gold) were prepared with an LKB Ultratome III and contrasted with the 3% uranyl acetate/lead citrate double-staining procedure [7]. Semithin plastic sections (1 μ) were prepared for light microscopy study by the polychromatic staining procedure of Ghidoni et al [8]. For comparison of techniques, specimens were also prepared for light microscopy with H&E, Warthin–Starry silver stain, periodic acid-Schiff (PAS), and tissue crush preparations stained with Giemsa stain.

Ultrastructural investigations were carried out with an RCA EMU-4 electron microscope.

RESULTS

Light Microscopy

The biopsied material (formalin fixed) stained with H&E contained no recognizable microorganisms, but the lesion was characterized by the presence of histiocytes/monocytes, polymorphonuclear leukocytes, and prominent microvascular structures. Donovan bodies were found within histiocyte-like cells following Warthin–Starry silver stain, as well as Giemsa staining of tissue crush preparations and touch imprints.

Material which had been prepared for electron microscopy by glutaraldehyde fixation and embedded in plastic revealed the presence of the organisms which were readily definable by light microscopy in semithick (1 μ) sections. Although the diagnostic value of plastic-embedded specimens in Donovanosis has been reported [3, 4], to our knowledge the superior information derived from polychromatic stains has not been described. With this staining procedure, the microorganisms stained as sharply defined, dark to pale reddish, ovoid structures which were within vacuolar re-

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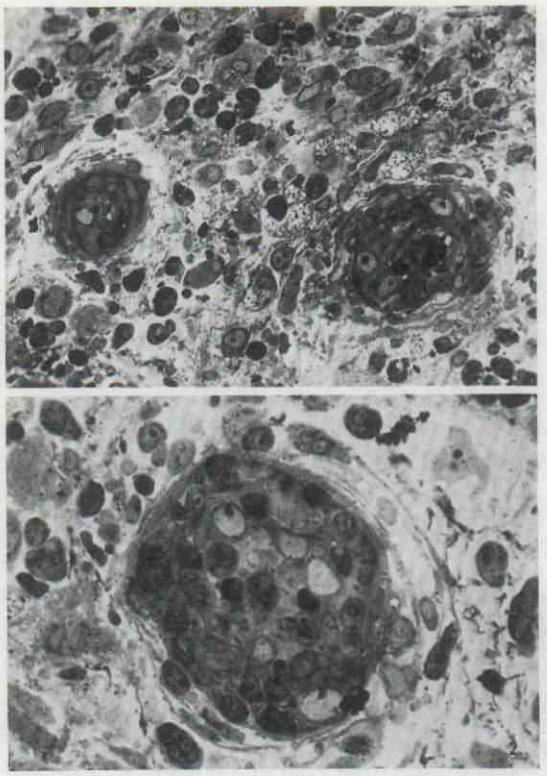

FIG. 1: Polychromatic stain of epoxy-embedded tissue demonstrating organisms in vacuoles in phagocytic cells. (× 420)

FIG. 2: Polychromatic stain demonstrating blood vessel with thickened cellular wall with lumen congested with neutrophils and other circulating cells. Numerous neutrophils are seen throughout wall of vessel as well. (× 420)

gions of the reactive cells. Variations in the "stainability" of the organism within a single vacuole may be related to the physiologic conditions of the individual organisms. The number of organisms within each vacuole varied greatly in a plane of section, from as few as 1 to as many as 20 or more. Although staining with toluidine blue is a more rapid procedure, we have found that the use of a polychromatic stain [8], though slightly more time consuming, offers even greater information than the monochromatic stains (Figs. 1, 2). The majority of the "phagocytic" cells contained a light purple karyoplasm which was surrounded by a darker-staining envelope region. Only occasionally did one encounter the organisms within the cytoplasm of polymorphonuclear cells.

The other formed elements within the tissue consisted of mononuclear cells which contained scanty cytoplasm, polymorphonuclear cells, a few erythrocytes, and fragments of connective tissue. The microvasculature consisted of thick-walled vessels with clearly defined endothelial cells and their surrounding perivascular structures (Fig. 2). Leukocytes were present in the walls and congested the lumens of some of the vessels.

Electron Microscopy

The organisms were predominantly in "histiocytic type" cells with only occasional microorganisms within a polymorphonuclear cell. The bacteria were enclosed within vacuolar compartments with the number of organisms per vacuole ranging from 1 to more than 20 (Fig. 3).

The phagocytic nuclei were irregular in shape with a homogeneously granular karyoplasm and peripherally clumped chromatin (Figs. 3, 4). Their cytoplasm was rich in mitochondria, rough endoplasmic reticulum, smooth endoplasmic reticulum, numerous Golgi complexes, and vesicular elements (Figs. 5, 6). Both microtubules and microfilaments were dispersed among the other organelles. The boundary of each vacuole was membrane-limited to varying degrees. In some

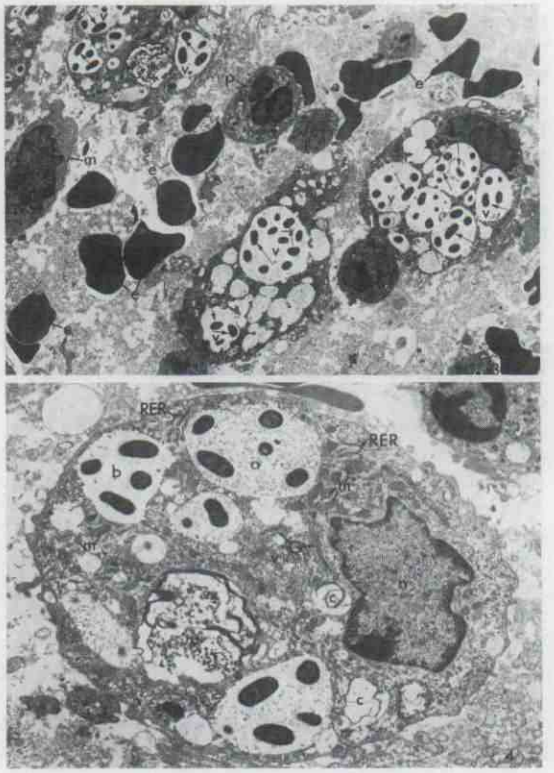

FIG. 3: The organisms (arrows) are shown within the vacuoles (v) of the phagocytic cells. Other elements within the amorphous matrix include erythrocytes (e), polymorphonuclear cells (p), and mononuclear cells (m). (× 1,300)

FIG. 4: The cellular components of this phagocyte —nucleus (n), mitochondria (m), Golgi (G), vesicles (v), rough endoplasmic reticulum (RER)—are evident. The variations in intravacuolar configurations which range from bacteria within granulated matrix (a) to bacteria in nongranulated vacuole (b) to intravacuolar micellular structures (c) are evident. (× 4,200)

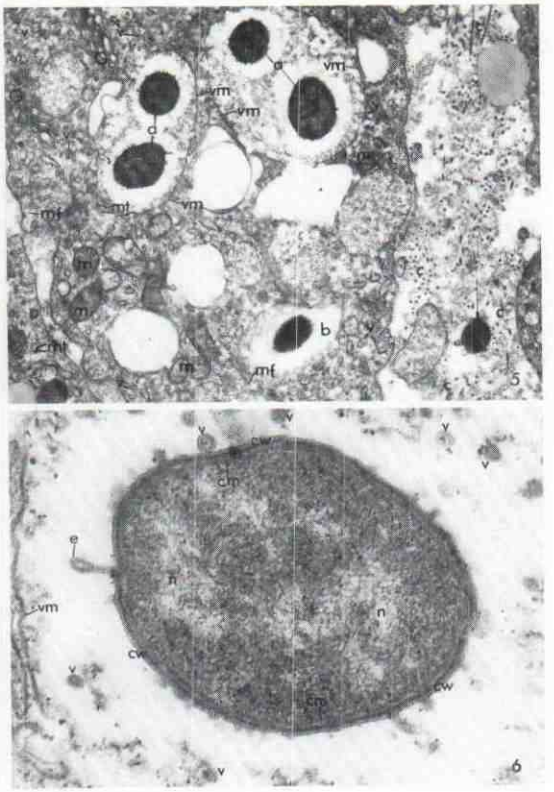

FIG. 5: The morphologic variations of microorganism/vacuolar relationships can be seen within the same phagocyte. Some organisms (a) have a clearly defined capsular area within the amorphous granulation of the vacuole whereas another organism (b) is within an agranular vacuole and has no obvious capsule. The difference in the vacuolar membrane (vm) of the separate vacuoles can also be seen between the one containing organisms (a) as compared with the lower vacuole with its organism (b). The cytoplasm of the phagocyte contains vesicles (v), Golgi complexes (G), and mitochondria (m). Microtubules (mt) and microfilaments (mf) are seen in the phagocyte. In the extracellular compartment, collagen fibers (c) are shown within the amorphous granular material as is one of the rarely encountered extraphagocytic organisms (arrow). (× 11,000)

FIG. 6: The vacuolar membrane (vm) separates the cytoplasm of the phagocyte from the agranular vacuole. The microorganism contains internal nuclear equivalent areas (n) and is limited in its outer surface by the cell membrane (cm) and cell wall (cw). Evaginations (e) are commonly seen from the cell wall and give rise to vesicles (v) which are characterized by a homogeneous central area surrounded by a double membrane of equivalent dimensions to that of the cell wall. (× 39,000)

vacuoles there was a broken membrane; in others, the vacuolar edge was defined by the granular surrounding cytoplasm with no limiting membrane being obvious (Fig. 5). The vacuoplasmic area contained amorphous material which, when sufficiently abundant, clearly outlined the bacterial cell wall/capsule area (Fig. 5). Other vacuoles (even within the same histiocyte) contained less dense granulation or a complete void of granulation and appeared electron lucent around the organism (Fig. 5). Through a selected plane of section, both electron-lucent or granulated vacuoles contained organisms. Still another type of vacuolar involvement consisted of the prevalence of a micellular type of structure which was similar to the reported "normal" and "abnormal" configurations of the lipid/glycoprotein type (9) (Figs. 4, 7). Other recognizable structures within the lesion included numerous polymorphonuclear cells, erythrocytes (Fig. 3), and scattered collagenous clumps (Figs. 4, 5), all of which were within a matrix of amorphous granular material.

In our investigation, the organisms were usually observed within cells. The bacterial ultrastructure consisted of an homogeneous, ovoid mass which contained internal areas of lighter homogeneity (nuclear equivalent areas) (Fig. 6). The organisms were limited by a cell membrane and the overlying cell wall. Evaginations from the cell wall were very common and were readily detectable and traceable

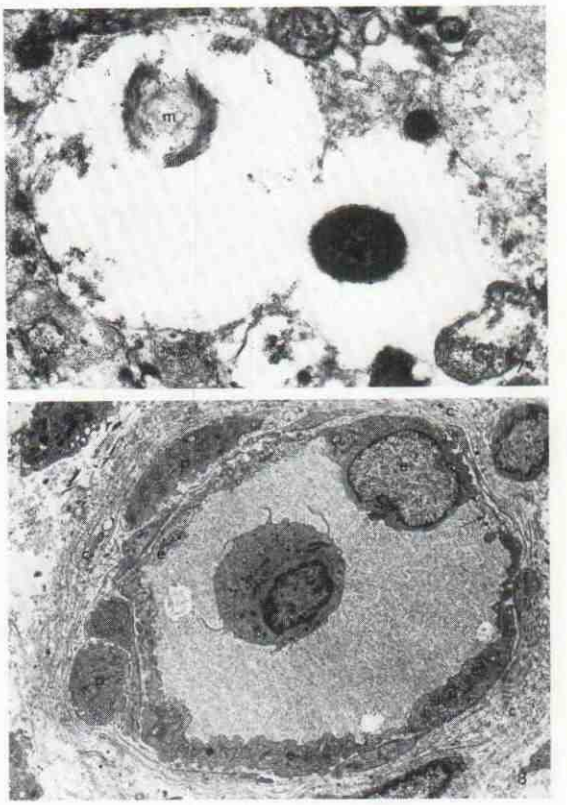

FIG. 7: Micellular structures (m) are seen within phagocytic vacuoles and are considered to represent degraded microorganisms. (× 13,000)

FIG. 8: The microvasculature within the lesion is characterized by prominent endothelial units (e) which are discontinuous at fenestrated areas (arrow) of the vessel wall. The endoplasmic reticulum is often distended, particularly within the pericytes (p). The vessels are surrounded by supportive connective tissue (c). (× 2,600)

from the unit structure of the cell wall (Fig. 6). Extra bacterial "blebs" or vesicles were also present and were formed from the cell wall. These structures contained homogeneous central regions surrounded by a double membranous coat (Fig. 6), and were similar to those reported in other papers [3, 4] as empty phage heads. In the present paper no intravesicular (extrabacterial) or intrabacterial granular structures were seen which could be classified as "phage-like" entities.

Some organisms displayed a sharply defined capsule while others had no clearly visible capsule (Figs. 4, 5). These conditions could be found within different vacuoles of the same histiocyte. Those organisms which appeared to be in a degradated or "ghost" state were without surrounding vacuolar granulation or capsule definition (Fig. 7).

Small filamentous projections were seen to extend from some of the microorganisms and were the reported size of bacterial fimbriae [10] or pili [11].

The microvasculature within the lesion was very prominent, with the continuity of the endothelial unit being broken by fenestrations (Fig. 8). Pericytes were commonly found associated with the vessels. The entire complex was enclosed in a circular mass of connective tissue within which could be found various interstitial cell types. In some vessels, the endothelium as well as the pericytic elements appeared to be swollen with distended endoplasmic reticulum and numerous vesicles (Fig. 8).

DISCUSSION

Plastic sections viewed by light microscopy could be analyzed to a greater extent than paraffin sections of the same material. Microorganisms within the specimens were found predominantly within large phagocytes which could best be described as "histiocytes," and they were rarely seen within polymorphonuclear cells. We found few organisms in the extracellular areas and felt this reflected a rather complete phagocytosis.

Ultrastructurally, the varying intravacuolar states of the organisms suggested varying degrees of degradation by the phagocytes. Since a close relationship exists in some pathogenic bacteria between the presence of a capsule and their virulence [12], the variations in capsular configurations may reflect attempts by the phagocytes to make these organisms avirulent.

We were unable to observe any structures which we could classify as "virus-like" particles. The cell wall evaginations and related vesicles could be "coreless virus" or "empty phage heads," but the lack of intrabacterial "phage-like" structures does not support such definitions. Without further biochemical and histochemical evidence, we must conclude that if any bacterial-related "viruses" exist, they are masked or "hidden" in this tissue. The reaction of the microvasculature does appear associated with the pathogenesis of the disease and should be further defined.

REFERENCES

1. Donovan C: Ulcerating granuloma of the pudenda. Indian Med Gaz 40:414, 1905
2. Anderson K, Demonreum W, Goodpasture E: An etiologic consideration of *Donovania granulomatis* cultivated from granuloma inguinale (three cases) in embryonic yolk. J Exp Med 81:25–40, 1945
3. Davis CM: Granuloma inguinale, a clinical histological, and ultrastructural study. JAMA 211:632–637, 1970
4. Davis CM, Collins C: Granuloma inguinale: an ultrastructural study of *Calymmatobacterium granulomatis*. J Invest Dermatol 53:315–321, 1969
5. Murray M: The use of epoxy resin-embedded tissues in histopathology. Science Tools 18:22–23, 1971
6. Erlandson RA: A new maraglas, D.E.R. 732 embedment for electron microscopy. J Cell Biol 22:704–709, 1964
7. Reynolds ES: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:209–212, 1963
8. Ghidoni JJ, Campbell MM, Adams JG, Thomas H, Ramos EE: A new multicolored staining procedure for one-micron sections of epoxy embedments. Electron Microscopy Soc Am Proc 29:240–241, 1968
9. Dodson RF, Wood JG: Concentric membranous bodies in central nervous tissue. Cytobios 7:61–69, 1973
10. Duguid JP, Wilkinson JF: Environmentally induced changes in bacterial morphology. Symp Soc Gen Microbiol 11:69–99, 1961
11. Brinton C: Mon-flagellar appendages of bacteriae. Nature (Lond) 183:782–786, 1959
12. Weibull C: Morphology and chemical anatomy of bacteria, Bacterial and Mycotic Infections of Man. Edited by RJ Dubos, JG Hirsch. Philadelphia, JB Lippincott, 1965, pp 37–71